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(54) Title: PEPTIDE ANTAGONISTS OF SH2 BINDING AND THERAPEUTIC USES THEREOF (57) Abstract Peptide compounds that antagonize the association of a protein tyrosin kinase with substrate regulatory protein and that incorporate the tyrosine autophosphorylation site of a receptor tyrosine kinase in its phosphorylated form, salts thereof, methods of production, pharmaceutical compositions containing said compounds and methods for treating proliferative disease using said compositions are disclosed.		

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PEPTIDE ANTAGONISTS OF SH2 BINDING AND
THERAPEUTIC USES THEREOF

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FIELD OF THE INVENTION

10 The present invention relates to novel peptide compounds, and pharmaceutical compositions containing the compounds as useful pharmacological agents in the control or treatment of proliferative disease such as cancer and psoriasis and in the control or treatment of viral, inflammatory, allergic, autoimmune, and cardiovascular disease. This invention also relates to methods for production of the compounds and methods of treatment employing the compounds.

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BACKGROUND OF THE INVENTION

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Many of the signal transduction pathways that regulate a variety of cellular processes including the differentiation and proliferation of normal and malignant cells operate through growth factor receptors or protooncogene encoded growth factor receptors which possess intrinsic tyrosine kinase activity [Reviews: Cell 61, 203 (1990); J. Biol. Chem. 265, 7709 (1990); Science 243, 1564 (1989)]. This class of receptors includes, but is not limited to, the epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), macrophage colony stimulating factor receptor (CSF-1R), and the platelet derived growth factor receptor (PDGFR). Growth factor (ligand) binding to the extracellular domain of these receptors results in propagation of a signal intracellularly via a sequence of events that includes: receptor autophosphorylation, activation of the receptor

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tyrosine kinase (TK), and the phosphorylation of endogenous regulatory (effector) proteins participating in the signal transduction pathway. Many of the effector proteins are enzymes and their catalytic activity is turned on or off according to their phosphorylation state. Therefore, the phosphorylation of effector proteins by tyrosine kinases regulates the signaling pathways controlling many cellular activities, including mitogenesis, cell growth, and differentiation.

Although all of the effector proteins that are substrates for the specific TKs are not known, the receptor binding sites for some have been identified. The receptor autophosphorylation sites serve as high affinity binding sites for substrate proteins containing a specific amino acid sequence called a Src-homology 2 (SH2) domain [Science 252, 668 (1991)]. SH2 domains have been shown to bind to protein sequences containing phosphotyrosine. SH2 domains are sequence regions of about 100 amino acids originally found in the noncatalytic region of the src family of cytoplasmic (nonreceptor) protein tyrosine kinases (PTKs). SH2 domains have also been identified in functionally unrelated cytoplasmic proteins which are substrates for the growth factor receptor TKs. These proteins are involved in cellular signaling and transformation and include, but are not limited to, the following: abl, bcr-abl, GRB2, phospholipase C γ 1 (PLC γ 1), ras GTPase activator protein (GAP), and the p85 subunit of phosphatidylinositol-3' kinase (PI3K).

Specific phosphotyrosine binding domains for SH2 containing proteins have been identified. For example, the tyrosine autophosphorylation sites in the COOH terminus of the EGFR [EMBO 9, 4375 (1990); PNAS U.S.A. 87, 8622 (1990)] and FGFR [Mol. Cell. Biol. 11,

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5068 (1991)] serve as the high affinity binding sites for PLC γ 1 and GAP. Similarly, for the PDGFR family of TKs, the tyrosine autophosphorylation sites in the kinase insert domain are binding sites for PI3K and GAP
5 [Mol. Cel. Biol. 11, 1125 (1991); EMBO 11, 1365 (1992)].

It has been shown that engineered receptors in which specific tyrosine autophosphorylation sites are lost through mutation (e.g., mutation of tyrosine to
10 phenylalanine) and/or truncation lose their ability to bind and phosphorylate specific SH2 containing substrates [for example see: EGFR/PLC: J. Biol. Chem. 267, 8672 (1992); EMBO 9, 3279 (1990)]. Nevertheless, some of these mutants retain full catalytic activity
15 and retain the ability to phosphorylate other cellular proteins. Likewise, a molecule capable of inhibiting the association with a specific kinase and a specific substrate, could selectively inhibit downstream signalling through a specifically targeted effector
20 protein.

It has been previously demonstrated that uncoupling a TK from the signal transduction pathway results therapeutically in antitumor activity. Antitumor activity for TK inhibitors has been
25 demonstrated both in vitro and in vivo [J. Antibiot. 39, 170 (1986); Eur. J. Cancer 26(6), 722 (1990); J. Med. Chem. 32, 2344 (1989); J. Med. Chem. 34, 1896 (1991); Cancer Res. 51, 4430 (1991); J. Med. Chem. 34, 2328 (1991); Helv. Chim. Acta. 75, 696 (1992); Cancer
30 Res. 52, 4492 (1992)].

For example, Yoneda, et al. (Cancer Res. 51, 4430 (1991)), have shown that tyrosine kinases inhibitors RS-13022 and 14620 suppressed EGF-stimulated proliferation of HER-14 cells (transfected NIH
35 3T3 cells) and MH-85 tumor cells in vitro. The MH-85 tumor is a well characterized human squamous cell

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carcinoma associated with three paraneoplastic syndromes: hypercalcemia, leukocytosis, and cachexia. MH-85 cells overexpress endogenous EGFR tyrosine kinase and are dependent on the EGFR signal transduction pathway for growth in vitro and in nude mice. In vivo, the compounds suppressed the growth of MH-85 tumors in nude mice as well as the expression of the paraneoplastic syndromes. An increase in life span of 75% was observed for RG-13022-treated tumor bearing mice.

It is alleged that the SH2 domain(s) in proteins mediate their selective binding to activated (autophosphorylated) TKs [See EMBO 11, 1365 (1992)]. Specific SH2 domains bind to certain protein amino acid sequence motifs at the receptor tyrosine autophosphorylation sites. The precise phosphotyrosine binding site or sites on TK receptors for some SH2 containing substrate proteins are known. For example, the EGFR phosphotyrosine high affinity binding site for the SH2 domains of PLC γ 1 has been identified as tyrosine (Tyr or Y) 992 [EMBO, 11(2), 559 (1992)]. For the FGF receptor (flg), Y766 was identified as the PLC-SH2 binding site [Molecular and Cellular Biology, 11(10), 5068-5078 (1991)].

For the two SH2 domains of PI3K, phosphorylated tyrosines Y751 (Y719 in murine PDGFR) and Y740 (Y708 in murine PDGFR) in the kinase insert domain of human PDGFR, have been identified as high and low binding sites, respectively [EMBO 11, 1373 (1992)]. Various synthetic peptides derived from the PDGFR incorporating phosphorylated tyrosine (pTyr) have been shown to specifically block the association of PI3K with the PDGFR [Molecular and Cellular Biology, 11(2), 1125-1132 (1991); Molecular and Cellular Biology, 12(4), 1451-1459 (1992); Cell 69, 413 (1992)]. These peptides, derived from an amino acid sequence in the PDGFR kinase insert domain incorporating PY708 and/or

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PY719, inhibit the ability of the receptor to bind to PI3K. They have no effect on the binding of other substrate proteins like PLC γ 1 or GAP. This is consistent with an earlier report that a PDGFR mutant with a deletion of the kinase insert domain could bind PLC γ 1 and c-raf-1 as efficiently as wildtype receptor, but could not bind PI3K kinase.

The peptides of this invention selectively inhibit the binding of SH2-containing substrate proteins to EGF receptor TK. Inhibition of this binding can block the ability of the TK to phosphorylate these specific substrate proteins and thereby inhibit specific downstream signal transduction pathways utilized in some hyperproliferative diseases like cancer.

SUMMARY AND DETAILED DESCRIPTION

We have unexpectedly found new peptides that have the ability to inhibit the association of a TK with SH2 containing substrate proteins. The peptides of this invention have been shown to selectively inhibit the binding of PLC γ 1-SH2 domains to the EGFR-TK or the abl-SH2 domain to the EGFR. Though not wishing to be bound by theory, this inhibition is probably the result of competitive inhibition wherein the phosphorylated peptide competes with phosphorylated receptor for the same binding site on the SH2 domain. Inhibition of this binding blocks the ability of the TK to phosphorylate the substrate protein. In so doing, the peptides can effectively uncouple the TK from the signal transduction pathway utilized by the effector protein. In particular, this invention pertains to the use of certain synthetic peptides to inhibit the binding of the SH2 domains of PLC γ 1 or abl to the EGF

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receptor and thereby inhibit subsequent cellular signal transduction pathways utilized in diseases such as viral, inflammatory, allergic, autoimmune, and cardiovascular diseases and in malignant diseases and other hyperproliferative disorders.

By physically preventing a kinase from associating with its substrates, the peptides of this invention uncouple the regulatory protein from its signalling cascade so that they have application for biological and therapeutic activity analogous to that of recognized tyrosine kinase inhibitors.

This invention therefore pertains in one preferred aspect to novel synthetic phosphotyrosine (pTyr represents tyrosine in which the 4'-phenolic hydroxyl is phosphorylated) containing peptides and their analogs derived from the primary amino acid sequence of the TK receptors. The peptides are "derived" from the TK receptors by the nature of their amino acid sequences. The peptides incorporate in their sequence the specific tyrosine that is the autophosphorylation site. The peptides amino acid sequence is derived from and incorporates the pTyr site of the TK that is selected from the EGFR, PDGFR, erbB2/neu, FGFR, or CSF1 tyrosine kinase.

Incorporation of a pTyr or a moiety "mimicking" pTyr within the peptide sequence is a critical element of the invention. A pTyr mimic being defined as a pTyr replacement resembling, yet distinct from, phosphotyrosine in one or more of its properties. Those properties in which a mimic could resemble phosphotyrosine could include, but are not limited to: electronic charge, size, hydrophobicity/hydrophilicity and/or pKa. The peptides of the invention inhibit the association of a protein tyrosine kinase with a protein that contains one or more SH2 domains that is selected from abl, bcr-abl, GRB2, PLC γ 1, GAP, and PI3K.

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More specifically, by the term "mimic", is meant a pTyr replacement distinct from pTyr but resembling pTyr in one or more of its properties and selected from phosphonomethyl-phenylalanine, phosphono(α -fluoro)-methylphenylalanine, phosphono(α,α -difluoro)-methylphenylalanine and phosphono(α -hydroxy)-methylphenylalanine, phosphono(α -chloro)methylphenylalanine, N-alkyl tyrosines, tyrosine sulfates, phosphonophenylalanine (J. Amer. Chem. Soc. 109, 2831 (1991)], negatively charged amino acids (for example aspartic and glutamic acids), phosphoserine, and phosphothreonine.

Specifically included in this invention are peptides incorporating an "unnatural" pTyr, or a pTyr in its protected form, or a pTyr mimic. For example, pTyr may be replaced with an enzymatically stable, nonhydrolyzable tyrosine phosphate moiety or Phe-derived mimic (Phe = phenylalanine) as described such as phosphonomethylphenylalanine and related derivatives [for description incorporated herewith by reference, see Tetrahedron Letters 32(43), 6061 (1991); Tetrahedron Letters 33(9), 1193 (1992); Synthesis 1019 (1991); Tetrahedron Letters 33(29), 4137 (1992); and Amer. Chem. Soc. 204th National Meeting, 122; J. Org. Chem. 58, 1336 (1993); Tetrahedron Letters 34(22), 3543 (1993)].

In addition, the invention includes peptide analogs modified by the replacement of some or all of the natural L aminoacids with the D aminoacid(s). Also included within the scope of this invention are N-terminally shortened fragments, C-terminally shortened fragments, and N-terminally and C-terminally shortened fragments containing pTyr or mimic thereof. N- and C-terminally modified derivatives include but are not limited to N-acetyl and/or carboxamide termini.

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Further, the invention includes the aforementioned phosphotyrosine containing peptides and their analogs which have undergone a mutation. A "mutation" in a peptide alters its primary structure (relative to the commonly occurring or specifically described peptide) due to changes in its synthetic preparation or the nucleotide sequence of the DNA which encodes it if prepared by recombinant technology. These mutations specifically include allelic variants. In spite of the mutation, the peptides and their analogs are still characterized by their function which is to inhibit the association of a protein tyrosine kinase with a protein that contains one or more SH2 domains that is selected from abl, bcr-abl, GRB2, PLC γ 1, GAP, and PI3K.

Mutational changes in the primary structure of a peptide result from deletions, additions, or substitutions. A "deletion" is defined as a polypeptide in which one or more internal amino acid residues are absent. An "addition" is defined as a polypeptide which has one or more additional internal amino acid residues as compared to the wild type. A "substitution" results from the replacement of one or more amino acid residues by other residues. A protein "fragment" is a polypeptide consisting of a primary amino acid sequence which is identical to a portion of the primary sequence of the protein to which the polypeptide is related.

Preferred "substitutions" are those which are conservative, i.e., wherein a residue is replaced by another of the same general type. As is well understood, naturally occurring amino acids can be subclassified as acidic, basic, neutral and polar, or neutral and nonpolar. It is generally preferred that peptides differing from the native form contain substituted amino acids, or codons for amino acids,

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which are from the same group as that of the amino acid replaced.

Thus, in general, the basic amino acid Lys, Arg, and His are interchangeable; the acidic amino acids aspartic and glutamic are interchangeable; the neutral polar amino acids Ser, Thr, Cys, Gln, and Asn are interchangeable; the nonpolar aliphatic acids Gly, Ala, Val, Ile, and Leu are conservative with respect to each other (but because of size, Gly and Ala are more closely related and Val, Ile and Leu are more closely related), and the aromatic amino acids Phe, Trp, and Tyr are interchangeable.

While proline is a nonpolar neutral amino acid, it represents difficulties because of its effects on conformation, and substitutions by or for proline are not preferred, except when the same or similar conformational results can be obtained. Polar amino acids which represent conservative changes include Ser, Thr, Gln, Asn; and to a lesser extent, Met. In addition, although classified in different categories, Ala, Gly, and Ser seem to be interchangeable, and Cys additionally fits into this group, or may be classified with the polar neutral amino acids. Some substitutions by amino acids, or codons for amino acids, from different classes may also be useful.

BRIEF DESCRIPTION OF THE TABLES

The invention is described by way of example with reference to the accompanying data Tables wherein:

Table 1 specifies a number of peptides which fall within the scope of the present invention;

Table 2 reports the peptide concentration (μM) which inhibits the equilibrium binding of phospholipase C γ 1 (PLC)NC*SH to activated EGF receptor by 50% (in

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each case the peptide is identified by its sequence ID number described herein below);

Table 3 reports the peptide concentration (μM) which inhibits the equilibrium binding of abl SH2 to activated EGF receptor by 50% (in each case identified by the number of its preparative example described herein below).

10 DETAILED DESCRIPTION OF THE INVENTION

The invention in one preferred aspect comprises peptide compounds in salt or nonsalt form having the formula

15
$$\text{X}-(\text{AA})_n-\text{Y} \quad \text{I}$$

selected from the following peptides, wherein the amino terminal X is NH_2- or $\text{CH}_3\text{CO}-\text{NH}$, the carboxy terminal Y is $-\text{COOH}$ or $-\text{CONH}_2$, AA is an amino acid, pTyr or Phe(X) as specified in TABLE 1 below, and n is an integer from 1-15, pTyr represents tyrosine in which the 4'-phenolic hydroxyl is phosphorylated and Phe(X) is a phospho-tyrosine mimic replacing pTyr in which the indicated functionality (X) is attached on the 4'-aromatic carbon of phenylalanine. A phosphotyrosine replacement may also be selected from but not limited to (phosphonomethyl)phenylalanine and related derivatives [namely phosphono(α -fluoromethyl)phenylalanine, phosphono(α,α -difluoromethyl)phenylalanine, di-O-(C_{1-4})alkyl-(α -fluoro)phosphono methylphenylalanine, di-O-(C_{1-4})-alkylphosphono(α,α -difluoro)methylphenylalanine, di-O-(C_{1-4} alkyl)phosphotyrosine, phosphono(α -hydroxymethyl)phenylalanine, phosphono(α -chloromethyl)phenylalanine], tyrosine thiophosphate, N-(C_{1-4})alkyl phosphotyrosines, tyrosine sulfates, 4'-phosphonophenylalanine, aspartic acid, glutamic acid, 35 phosphoserine, and phosphothreonine.

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TABLE 1
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AA=	Example	Seq ID No.
Val-Val-Asp-Ala-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	1	1
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	2	2
Phe-Leu-Pro-Val-Pro-Glu-pTyr-Ile-Asn-Gln-Ser-Val	3	3
Asp-Asn-Pro-Asp-pTyr-Gln-Gln-Asp-PhePhe	4	4
Ser-Leu-Asp-Asn-Pro-Asp-pTyr-Gln-Gln-Asp-Phe-Phe	5	5
Ala-Glu-pTyr-Leu-Arg-Val-Ala-Pro-Gln-Ser	6	6
Thr-Ala-Glu-Asn-Ala-Glu-pTyr-Leu-Arg-Val-Ala-Pro-Gln-Ser	7	7
Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Gln-Gly	8	8
Ser-Pro-Gln-Pro-Glu-pTyr-Val-Asn-Gln-Ser-Gly	9	9
Asp-Asn-Leu-pTyr-Tyr-Trp-Asn-Gln-Ser-Ser	10	10
Asp-Asn-Leu-Tyr-pTyr-Trp-Asp-Gln-Ser-Ser	11	11
Glu-Asn-Pro-Glu-pTyr-Leu-Gly-Leu-Asp-Val-Pro-Val	12	12
Leu-Thr-Ser-Asn-Gln-Glu-pTyr-Leu-Asp-Leu-Ser-Met-Pro-Leu	13	13
Glu-pTyr-Cys-Tyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Glu-Gln	14	14
Glu-Tyr-Cys-pTyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Glu-Gln	15	15
Ala-Glu-pTyr-Leu-Arg-Val-Ala	16	16
pTyr-Leu-Arg-Val-Ala-Pro-Gln-Ser	17	17
Ala-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	18	18
Asp-Ala-Ala-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	19	19
Asp-Ala-Asp-Ala-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	20	20
Asp-Ala-Asp-Glu-pTyr-Ala-Ile-Pro-Gln-Gln-Gly	21	21
Asp-Ala-Asp-Glu-pTyr-Leu-Ala-Pro-Gln-Gln-Gly	22	22
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Ala-Gln-Gln-Gly	23	23
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Ala-Gln-Gly	24	24
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Ala-Gly	25	25
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Ala	26	26
Leu-Pro-Val-Pro-Glu-pTyr-Ile-Asn-Ala-Ser-Val	27	27
Glu-pTyr-Leu	28	28
Asp-Glu-pTyr-Leu-Ile	29	29
Ala-Ala-Asp-Ala-pTyr-Ala-Ile-Pro-Ala-Ala	30	30
Asp-Ala-pTyr-Ala-Ile	31	31

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TABLE 1
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AA=	Example	Seq ID No.
Val-Val-Asp-Ala-Asp-Glu-pTyr-Leu		32
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly	23	33
$ \begin{array}{c} \text{O} \\ \\ \text{CH}_2\text{-P-OMe} \\ \\ \text{OMe} \end{array} $		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		34
$ \begin{array}{c} \text{O} \\ \\ \text{CH}_2\text{-P-OtBu} \\ \\ \text{OtBu} \end{array} $		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		35
$ \begin{array}{c} \text{O} \\ \\ \text{CF}_2\text{-P-OtBu} \\ \\ \text{OtBu} \end{array} $		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		36
$ \begin{array}{c} \text{O} \\ \\ \text{CHF-P-OtBu} \\ \\ \text{OtBu} \end{array} $		

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TABLE 1
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AA=	Example	Seq ID No.
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly	24	37
$ \begin{array}{c} \text{O} \\ \\ \text{CH}_2\text{-P-OH} \\ \\ \text{OH} \end{array} $		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		38
$ \begin{array}{c} \text{O} \\ \\ \text{CF}_2\text{-P-OH} \\ \\ \text{OH} \end{array} $		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		39
$ \begin{array}{c} \text{O} \\ \\ \text{CHF-P-OH} \\ \\ \text{OH} \end{array} $		
Leu-Val-Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Glu-Gly		40
Asp-Ala-Asp-Glu-pTyr		41
Ala-Asp-Glu-pTyr-Leu-Ile		42
Ala-Asp-Glu-pTyr-Leu-Ile-Pro		43
Asp-Ala-Ala-Glu-pTyr-Leu-Ala-Ala-Gln-Gln-Gly	33	44
Ala-Ala-Asp-Ala-pTyr-Ala-Ile-Pro-Ala-Ala-Ala	34	45
Asp-Glu-pTyr-Leu		46
Glu-pTyr-Leu-Ile		47
Asp-Glu-pTyr		48
Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Gln-Gly		49
Ser-Pro-Gln-Pro-Glu-pTyr-Val-Asn-Gln-Ser-Glu		50
Glu-pTyr-Cys-Tyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Glu		51
Glu-Tyr-Cys-pTyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Gln		52

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TABLE 1
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AA=	Example	Seq ID No.
Gly-Ser-Val-Gln-Asn-Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	25	53
Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn		54
Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	35	55
Gly-Ser-Val-Gln-Asn-Pro-Val-pTyr-Glu-Asn-Val		56
Pro-Val-pTyr-Glu-Asn-Val		57
pTyr-Ala-Ala-Val	30	58
Leu-Val-Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Gln-Gly		59
Asp-Ala-Asp-Glu-pTyr	27	60
Ala-Asp-Glu-pTyr-Leu-Ile	28	61
Ala-Asp-Glu-pTyr-Leu-Ile-Pro	29	62
Asp-Ala-Ala-Glu-pTyr-Leu-Ala-Ala-Glu-Glu-Gly	30	63
Ala-Ala-Asp-Ala-pTyr-Ala-Ile-Pro-Ala-Ala-Ala	31	64
Asp-Glu-pTyr-Leu-Ile	32	65
Asp-Glu-pTyr-Leu	36	66
Glu-pTyr-Leu-Ile	37	67
Val-Gln-Asn-Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	38	68
Asn-Pro-Val-pTyr-Asn-Gln-Pro-Leu-Asn	39	69
Asn-Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn		70
Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	40	71
Pro-Val-pTyr-Glu-Asn-Pro-Pro-Leu-Asn	41	72
Asp-Ala-Asp-Glu-pTyr-Leu	42	73
Asp-Ala-Glu-pTyr-Leu	43	74
Pro-Val-pTyr-Glu-Asn-Pro-Pro-Leu-Asn		75

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The compounds of the invention are capable of further forming both pharmaceutically acceptable acid addition and/or base salts depending on the particular amino acid sequence. All these forms are within the scope of the present invention. The compounds of the invention possess chiral centers and each center may exist in the (D) or (L) configuration. The present invention includes, therefore, all enantiomeric and diastereomeric forms as well as the appropriate mixtures thereof. In addition, this invention also includes peptides truncated at the N-terminus, C-terminus, or both the N- and C-termini whose total length is no less than two amino acids.

The compounds of Formula I are valuable inhibitors of the association of a tyrosine kinase with its cellular substrates and effectively uncouple the tyrosine kinase from specific signal transduction pathway(s).

The tests employed indicate that compounds of Formula I possess the inhibiting activity. Thus, the compounds were tested in a biological assay described herein for their ability to inhibit binding.

The compounds in pharmaceutical composition form may be administered orally or parenterally or by direct injection into the target organ. The usual human dosage ranges for a 80 kg subject from about 1 mg to about 1 g per day (0.01 mg to 10 mg per kg of weight per day), preferably 10 mg to 100 mg per day (0.1 mg to 1.0 mg per kg of weight per day), optionally in divided portions.

The above employed pharmaceutical compositions are produced by formulating a compound of the foregoing formula (active ingredient) in dosage unit form with a pharmaceutical carrier. Some examples of dosage unit forms are tablets, capsules, lozenges, and pills; as well as powders and aqueous and nonaqueous oral

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solutions and suspensions and parenteral solutions, packaged in containers containing either one or some larger number of dosage units and capable of being subdivided into individual doses by such means as measurement into a teaspoon or other standard container. Some examples of suitable pharmaceutical carriers, including pharmaceutical diluents, are gelatin capsules; sugars such as lactose and sucrose; starches such as corn starch and potato starch; cellulose derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, methyl cellulose, and cellulose acetate phthalate; gelatin; talc, stearic acid; magnesium stearate; vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil, and oil of theobroma; propylene glycol; glycol; glycerine, sorbitol; polyethylene glycol; water; agar; alginic acid; isotonic saline, and phosphate buffer solutions; as well as other compatible substances normally used in pharmaceutical formulations. The compositions of the invention can also contain other components such as coloring agents, flavoring agents, and/ or preservatives. These materials, if present, are usually used in relatively small amounts. The compositions can, if desired, also contain other therapeutic agents.

The percentage of the active ingredient in the foregoing compositions can be varied within wide limits but for practical purposes it is preferably present in a concentration of at least 10% in a solid composition and at least 2% in a primarily liquid composition. The most satisfactory compositions are those in which a much higher proportion of the active ingredient is present. The compositions of the invention preferably contain from 2 mg to 1.0 g of the active ingredient per dosage unit so that the entire amount to be

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administered during a day can be made up from a reasonable number of dosage units.

In a preferred aspect the invention comprises a method useful for treating proliferative disease or for
5 treating viral, inflammatory, allergic, or cardiovascular disease in a mammal with a composition containing a therapeutically effective amount of a peptide in pharmaceutically acceptable salt form or nonsalt form according to the invention.

10 Among the most preferred compounds in this invention for inhibiting the association of the PLC γ 1 SH2 domains with the EGFR are those incorporating the Y992 position of the EGFR, the Y1086 position of the EGFR or the Y351 position in the neu receptor.

15 Biological Assay. A bacterial glutathione-S-transferase fusion protein, containing both SH2 domains of PLC γ 1, was labelled in vivo with ^{35}S . Phosphorylated EGFR was purified from EGF-treated
20 3T3 cells transfected with the human EGFR by precipitation with anti-EGFR antisera. The resulting immune complex was bound to protein A - Sepharose beads. Binding of the ^{35}S labelled PLC γ 1 fusion protein (25 nM) to the purified EGFR-beads complex
25 (approximately 1 μM of receptor/assay) was allowed to proceed to equilibrium (20 min) in a total volume of 0.4 mL in the presence of the various concentrations of peptides. Bound ^{35}S fusion protein was separated from free by centrifugation, and radioactivity was
30 determined by scintillation counting.

Results reported below in Table 2 represent the concentration of peptide (micromolar) at which equilibrium binding is inhibited by 50% and are obtained from the mean of triplicate determinations \pm
35 standard deviations repeated in five separate experiments. These results show that representative

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peptides of the invention were effective in inhibiting the binding of both SH2 domains of PLC γ 1 to the EGF receptor.

In a similar fashion, a bacterial glutathione-S-transferase fusion protein, containing the SH2 domain of abl, was labelled in vivo with ^{35}S and used in the assay described above in place of the PLC γ 1 fusion protein. The assay was used to evaluate the inhibition of the binding of a regulatory protein containing the SH2 domain of abl with the EGF receptor by certain peptides of the present invention. The results of this inhibition study are reported below in Table 3.

TABLE 2. Inhibition of the Binding of PLC NC*SH Activated EGF Receptor by Identified Peptides

Seq ID	IC ₅₀ (μM)
2	8
3	26
16	47
18	7.8
20	15
21	18
24	22
25	6.5
26	13

TABLE 3. Inhibition of the Binding of abl SH2 to Activated EGF Receptor by Identified Peptides

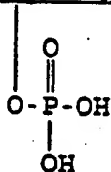
Seq ID	IC ₅₀ (μM)
53	34
57	45

The invention and modes of practicing the same are described in the following nonlimitative examples.

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EXAMPLE 1

Asp-Ala-Asp-Glu-Tyr-Leu-Ile-Pro-Gln-Gln-Gly-(OH)

Seq ID No:2

10 The title compound was prepared, in its
unphosphorylated form, by standard solid phase
synthetic peptide methodology utilizing a
fluorenylmethoxycarbonyl (Fmoc)/tertiary-butyl (t-Bu)
strategy (J. M. Stewart and J. D. Young, Solid Phase
15 Peptide Synthesis, Pierce Chemical Co., Rockford, IL,
1984), with the two following exceptions. First, the
tyrosine was incorporated with the phenolic hydroxy
group unblocked, so as to render it available for
phosphorylation on the resin. Second, the terminal Asp
20 was incorporated as its N-tertiary-butoxycarbonyl
(t-Boc) derivative such that cleavage of this
N-terminal protecting group could be achieved
concomitantly with cleavage of the peptide from the
resin rather than require a separate cleavage step.
25 All protected amino acids and reagents were obtained
from commercial sources except where noted and were not
further purified. The protected peptide resin was
prepared on an Applied Biosystems 431A Peptide
Synthesizer, utilizing protocols supplied for an
30 hydroxybenzotriazole (HOBt) activated ester coupling
scheme (Standard Fmoc, Version 1.12). The synthesis
was commenced by loading 0.25 mmol (0.29 g,
0.85 mmol/g) of Wang resin (4-alkoxybenzyl alcohol
resin) with 0.5 mmol of N-Fmoc-Gly symmetrical
35 anhydride using 4-dimethylaminopyridine as catalyst.
The protected peptide was prepared by the sequential
coupling of the following amino acids (in order of
addition): N-Fmoc-Gln, N-Fmoc-Gln, N-Fmoc-Pro,

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5 N-Fmoc-Ile, N-Fmoc-Leu, N-Fmoc-Tyr, N-Fmoc-Glu(O-t-Bu),
N-Fmoc-Asp(O-t-Bu), N-Fmoc-Ala, N-t-Boc-Asp(O-t-Bu). A
typical cycle for the coupling of an individual amino
acid residue is illustrated below (reproduced from the
ABI manual):

1. Deprotection with 20% piperidine in
N-methylpyrrolidone (NMP), 21 minutes
2. Washes with nmp, 9 minutes
- 10 3. Coupling of the HOBT activated ester in nmp,
71 minutes
4. Washes with nmp, 7 minutes

15 After coupling the last amino acid, the protected
peptide resin was dried at reduced pressure to give
0.701 g of material. The tyrosine was phosphorylated
according to the procedure of G. Staerkaer;
M. H. Jakobsen; C. E. Olsen; A. Holm. Solid Phase
Peptide Synthesis of Selectively Phosphorylated
20 Peptides. Tetrahedron Letters 5389-5392 (1991). Thus,
0.35 g (0.12 mmol) of the protected peptide resin was
added to a rocking reaction vessel and washed with
dimethylformamide (DMF) (3 x 15 mL), dichloromethane
(DCM) (3 x 15 mL), and dry tetrahydrofuran (THF) (3 x
25 15 mL, newly opened bottle of anhydrous material,
Aldrich). To the resin was added 12 mL of dry THF,
followed by 1.56 g (6.25 mmol, 50 equiv.) of
di-tertiary-butyl-N,N-diethylphosphoramidate (Perich,
J.W.; Johns, R.B. Di-tert-butyl
30 N,N-Diethylphosphoramidate. A New Phosphitylating
Agent for the Efficient Phosphorylation of Alcohols.
Synthesis 142-144 (1988) and then 1.32 g (18.8 mmol,
150 equiv.) of tetrazole. The suspension was rocked
for 60 minutes, then drained, and washed with dry THF
35 (3 x 15 mL), THF (10 x 15 mL, reagent grade (not
dried)), and DCM (7 x 15 mL). The resin was suspended

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in 12 mL of DCM and treated with 0.34 mL (2.5 mmol, 20 equiv.) of a 70% solution of tert-butyl hydroperoxide in water and rocked for 60 minutes. The suspension was drained, and the resin was washed with DCM (5 x 15 mL), DMF (5 x 15 mL), and finally DCM (5 x 15 mL), and dried under reduced pressure to give 359 mg of material.

The peptide was liberated from the solid support and the side chains deblocked by treatment of the 359 mg of the protected peptide resin with a solution of 9.5 mL of trifluoroacetic acid (TFA) and 0.5 mL of H₂O for 2 hours at room temperature. The spent resin was collected by filtration and discarded, and the filtrate was concentrated under reduced pressure, diluted with H₂O, and lyophilized to give 195 mg of a white solid. The crude peptide (150 mg) was dissolved in 2 mL of 50% acetic acid/H₂O, filtered through a 0.45 μ M syringe filter, and chromatographed on a Vydac 218TP 1022 column (2.2 x 25.0 cm, 15.0 mL/min, A: 0.1% TFA/H₂O, B: 0.1% TFA/CH₃CN; gradient of 0% B for 10 minutes, 0% to 8% B during 10 minutes, and 8% to 28% B during 120 minutes). Individual fractions were collected and combined based upon analysis by analytical HPLC. The combined fractions were concentrated under reduced pressure, diluted with H₂O, and lyophilized to give 81 mg of the product as a white solid. The homogeneity and structure of the resulting peptide was confirmed by analytical HPLC, capillary zone electrophoresis, proton nuclear magnetic resonance spectroscopy (¹H-NMR and ³¹P-NMR), amino acid analysis, and electrospray mass spectroscopy (ES-MS) (MH⁺ calcd., 1328.5; Found, 1328.8).

In a process analogous to Example 1, except where noted, using the appropriate amino acids, the following compounds were prepared.

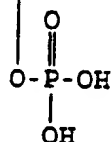
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EXAMPLE 2

Phe-Leu-Pro-Val-Pro-Glu-Tyr-Ile-Asn-Gln-Ser-Val-(OH)

5



Seq ID No:3

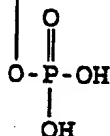
10

This peptide was liberated from the resin and deblocked by treatment as in Example 1 but for 7 hours. ES-MS, MH^+ calcd., 1485.7; Found, 1485.1.

EXAMPLE 3

Asp-Asn-Pro-Asp-Tyr-Gln-Gln-Asp-Phe-Phe-(OH)

20



Seq ID No:4

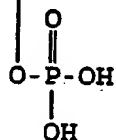
25

This peptide was liberated from the resin and deblocked by treatment as in Example 1 but for 18 hours. Fast Atom Bombardment Mass Spectroscopy (FAB-MS), $(\text{M}+\text{Na})^+$ calcd., 1390.5; Found, 1391.3.

EXAMPLE 4

Ala-Glu-Tyr-Leu-Arg-Val-Ala-Pro-Gln-Ser-(OH)

30



Seq ID No:6

35

This peptide was liberated from the resin and deblocked by treatment with a solution of 10 mL of TFA, 0.5 mL of thioanisole, 0.25 mL of ethanedithiol, 0.75 g of crystalline phenol, and 0.5 mL of H_2O for 4.5 hours at room temperature. FAB-MS, MH^+ calcd., 1213.5; Found, 1213.9.

40

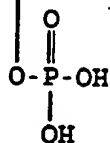
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EXAMPLE 5

Asp-Ala-Glu-Glu-Tyr-Leu-Val-Pro-Gln-Gln-Gly-(OH)

5



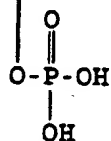
Seq ID No:8

10

This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 1350.5; Found, 1350.8.

EXAMPLE 6

15

Ser-Pro-Gln-Pro-Glu-Tyr-Val-Asn-Gln-Ser-Gly-(OH)

Seq ID No:9

20

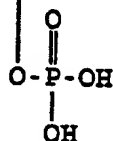
This peptide was liberated from the resin and deblocked by treatment as in Example 1 but for 2 hours. FAB-MS, (M+Na)⁺ calcd., 1286.2; Found, 1286.2.

25

EXAMPLE 7

Asp-Asn-Leu-Tyr-Tyr-Trp-Asp-Gln-Asn-Ser-Ser-(OH)

30



Seq ID No:10

35

This peptide was liberated from the resin and deblocked by treatment with a solution of 9.5 mL of TFA, 0.5 mL of H₂O, and 0.5 mL of ethanedithiol for 90 minutes at room temperature. ¹H-NMR, ³¹P-NMR, and amino acid analysis consistent with structure.

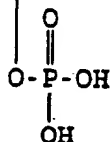
40

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EXAMPLE 8

Asp-Asn-Leu-Tyr-Tyr-Trp-Asp-Gln-Asn-Ser-Ser-(OH)

5



Seq ID No:11

10

This peptide was liberated from the resin and deblocked by treatment with a solution of 9.5 mL of TFA, 0.5 mL of H₂O, and 0.5 mL of ethanedithiol for 90 minutes at room temperature. ¹H-NMR, ³¹P-NMR, and amino acid analysis consistent with structure.

15

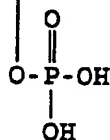
Analysis calculated for C₆₂H₈₂N₁₅O₂₆P.1 TFA.10 H₂O:

C, 43.22; H, 5.27; N, 11.81;

Found: C, 43.27; H, 4.91; N, 11.04.

EXAMPLE 9

20

Glu-Asn-Pro-Glu-Tyr-Leu-Gly-Leu-Asp-Val-Pro-Val-(OH)

Seq ID No:12

25

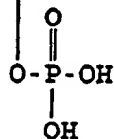
This peptide was liberated from the resin and deblocked by treatment as in Example 1. ES-MS, MH⁺ calcd., 1425.8; Found, 1425.5.

30

EXAMPLE 10

Ala-Glu-Tyr-Leu-Arg-Val-Ala-(OH)

35



Seq ID No:16

40

-25-

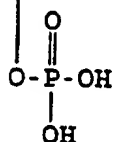
This peptide was liberated from the resin and deblocked by treatment as in Example 3 but for 3.5 hours. ES-MS, MH^+ calcd., 901.4; Found, 901.3.

5

EXAMPLE 11

Tyr-Leu-Arg-Val-Ala-Pro-Gln-Ser- (OH)

10



Seq ID No:17

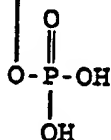
This peptide was liberated from the resin and deblocked as in Example 5. ES-MS, MH^+ calcd., 1013.5; Found, 1012.3.

15

EXAMPLE 12

Ala-Ala-Asp-Glu-Tyr-Leu-Ile-Pro-Gln-Gln-Gly- (OH)

20



Seq ID No:18

25

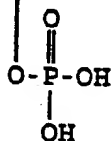
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, $(M+Na)^+$ calcd., 1307.3; Found, 1307.2.

30

EXAMPLE 13

Asp-Ala-Ala-Glu-Tyr-Leu-Ile-Pro-Gln-Gln-Gly- (OH)

35



Seq ID No:19

40

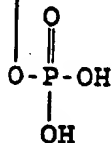
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, $(M+Na)^+$ calcd., 1306.8; Found, 1307.2.

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EXAMPLE 14

Asp-Ala-Asp-Ala-Tyr-Leu-Ile-Pro-Gln-Gln-Gly-(OH)

5



Seq ID No:20

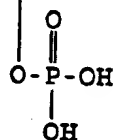
10

This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, MH^+ calcd., 1270.3; Found, 1270.3.

EXAMPLE 15

Asp-Ala-Asp-Glu-Tyr-Ala-Ile-Pro-Gln-Gln-Gly-(OH)

20



Seq ID No:21

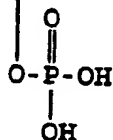
25

This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, MH^+ calcd., 1287.5; Found, 1287.6.

EXAMPLE 16

Asp-Ala-Asp-Glu-Tyr-Leu-Ala-Pro-Gln-Gln-Gly-(OH)

30



Seq ID No:22

35

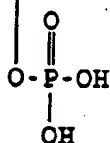
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, MH^+ calcd., 1286.5; Found, 1287.1.

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EXAMPLE 17

Asp-Ala-Asp-Glu-Tyr-Leu-Ile-Ala-Gln-Gly-(OH)

5



Seq ID No:23

10

This peptide was liberated from the resin and deblocked by treatment as in Example 1. Amino acid analysis consistent with structure.

Analysis calculated for C₅₂H₈₀N₁₃O₂₄P.1 TFA.9 H₂O:

C, 41.09; H, 6.32; N, 11.54;

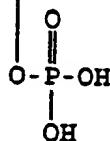
15

Found: C, 41.05; H, 5.53; N, 10.81.

EXAMPLE 18

Asp-Ala-Asp-Glu-Tyr-Leu-Ile-Pro-Ala-Gln-Gly-(OH)

20



Seq ID No:24

25

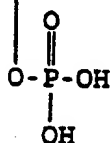
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 1293.3; Found, 1293.3.

30

EXAMPLE 19

Asp-Ala-Asp-Glu-Tyr-Leu-Ile-Pro-Gln-Ala-Gly-(OH)

35



Seq ID No:25

40

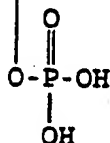
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, MH⁺ calcd., 1271.5; Found, 1271.5.

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EXAMPLE 20

Asp-Ala-Asp-Glu-Tyr-Leu-Ile-Pro-Gln-Gln-Ala-(OH)

5



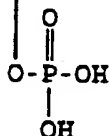
Seq ID No:26

10

This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, MH^+ calcd., 1342.6; Found, 1342.6.

EXAMPLE 21

15

Leu-Pro-Val-Pro-Glu-Tyr-Ile-Asn-Ala-Ser-Val-(OH)

Seq ID No:27

20

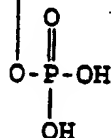
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, MH^+ calcd., 1282.4; Found, 1282.7.

25

EXAMPLE 22

Glu-Tyr-Leu-(OH)

30



Seq ID No:28

35

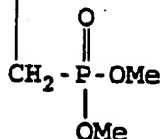
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, MH^+ calcd., 504.4; Found, 504.3.

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EXAMPLE 23

Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly-(OH)

5



Seq ID No:33

10 The title compound was prepared by standard solid phase synthetic peptide methodology utilizing a t-Boc/benzyl (Bzl) strategy. The N-t-Boc-p-dimethylphosphonomethyl-L-phenylalanine was prepared as described (M. Cushman; E. S. Lee.

15 Preparation of an Angiotensin I Analog Containing a p-Phosphonomethyl-L-phenylalanine Residue Via Asymmetric Synthesis of t-Boc-pDimethylphosphonomethyl-L-phenylalanine. Tetrahedron Letters 1193-1196 (1992). The protected peptide resin was prepared on an Applied Biosystems

20 431A Peptide Synthesizer, utilizing protocols supplied for an HOBT activated ester coupling scheme (Standard Boc, Version 1.12). The protected peptide was prepared by the sequential coupling of the following amino acids

25 (in order of addition) to 0.50 mmol (0.707 g, 0.707 mmol/g) of N-Boc-Gly phenylacetamido- methyl (PAM) resin: N-Boc-Gln, N-Boc-Gln, N-Boc-Pro, N-Boc-Ile, N-Boc-Leu, N-Boc-p-dimethylphosphono-

30 methyl-L- phenylalanine, N-Boc-Glu(OBzl), N-Boc-Asp(0-cyclohexyl), N-Boc-Ala, and N-Boc-Asp(0-cyclohexyl). A typical cycle for the coupling of an individual amino acid is illustrated below (reproduced from the ABI manual):

- 35 1. Deprotection with 25% TFA/DCM, 3 minutes
2. Deprotection with 50% TFA/DCM, 16 minutes
3. Washes with DCM, 3 minutes

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4. Washes with 5% diisopropylethylamine (DIEA)/DCM, 4 minutes
5. Washes with nmp, 5 minutes
6. Coupling of the HOBT activated ester in nmp, 39 minutes
7. Addition of DMSO to make 15% DMSO/85% nmp, 16 minutes
8. Addition of 3.8 equiv of DIEA, 5 minutes
9. Washes with nmp
10. Capping with 10% acetic anhydride, 5% DIEA in nmp, 9 minutes
11. Washes with DCM, 4 minutes

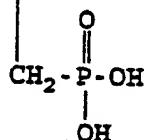
After the terminal amino acid had been coupled, the N-terminal Boc group was removed, and the protected peptide resin was washed with DCM and dried at reduced pressure to give 1.222 g of material. The peptide was liberated from the solid support and the side chains deblocked by treatment of 0.60 g of the material with hydrogen fluoride (HF)/p-cresol (10 mL/1 mL) at 0°C for 1 hour. The HF was removed at 0°C by a stream of nitrogen. The peptide and resin were triturated with ether and collected by filtration. The peptide was extracted with 50% AcOH/H₂O (4 x 25 mL), and the extracts were concentrated under reduced pressure, diluted with H₂O, and lyophilized to give 225 mg of a white solid. Preparative HPLC gave the title compound. FAB-MS, (M+Na)⁺ calcd., 1376.6; Found, 1376.2.

30

EXAMPLE 24

Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly-(OH)

35



Seq ID No:37

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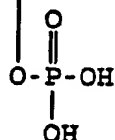
-31-

Treatment of 20 mg of the peptide in Example 23 at 25°C with a solution of 1.0 mL TFA, 0.6 mL dimethylsulfide, 0.2 mL m-cresol, and 0.2 mL trifluoromethanesulfonic acid gave the title compound.

5 FAB-MS, (M+H)⁺ calcd., 1326.6; Found, 1326.6.

EXAMPLE 25

Gly-Ser-Val-Gln-Asn-Pro-Val-Tyr-His-Asn-Gln-Pro-Leu-
10 Asn- (OH)



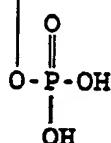
Seq ID No:53

15 This peptide was liberated from the resin and deblocked by treatment with a solution of 9.5 mL of TFA, 0.25 mL of 1,2-ethanedithiol, and 0.25 mL of water for 2 hours at room temperature. ES-MS, (M+H)⁺ calcd.,

20 1647.7; Found, 1647.5.

EXAMPLE 26

H-Tyr-Ala-Ala-Val- (NH₂)

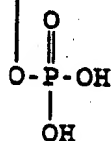


Seq ID No:58

30 This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 500.5; Found, 501.8.

EXAMPLE 27

Asp-Ala-Asp-Glu-Tyr- (NH₂)



Seq ID No:60

SUBSTITUTE SHEET

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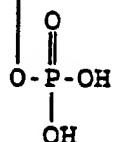
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 690.6; Found, 690.2.

5

EXAMPLE 28

Ala-Asp-Glu-Tyr-Leu-Ile-(NH₂)

10



Seq ID No:61

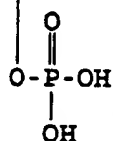
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 801.8; Found, 801.4.

15

EXAMPLE 29

Ala-Asp-Glu-Tyr-Leu-Ile-Pro-(OH)

20



Seq ID No:62

25

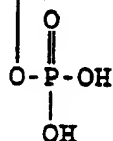
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 900.5; Found, 900.5.

30

EXAMPLE 30

Asp-Ala-Ala-Glu-Tyr-Leu-Ala-Ala-Glu-Gly-(OH)

35



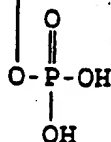
Seq ID No:63

-33-

EXAMPLE 31

Ala-Ala-Asp-Ala-Tyr-Ala-Ile-Pro-Ala-Ala-Ala-(OH)

5



Seq ID No:64

10

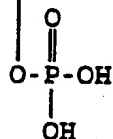
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 1083.1; Found, 1084.5.

EXAMPLE 32

15

Asp-Glu-Tyr-Leu-Ile-(OH)

20



Seq ID No:65

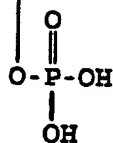
25

This peptide was liberated from the resin and deblocked by treatment as in Example 1.

EXAMPLE 33

Asp-Ala-Ala-Glu-Tyr-Leu-Ala-Ala-Gln-Gln-Gly-(OH)

30



Seq ID No:44

35

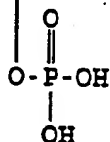
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 1214.2; Found, 1217.3.

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EXAMPLE 34

Ala-Ala-Asp-Ala-Tyr-Ala-Ile-Pro-Ala-Ala-Ala-(OH)

5



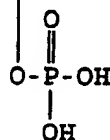
Seq ID No:45

10

This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 1083.1; Found, 1084.5.

EXAMPLE 35

15

Val-Tyr-His-Asn-Gln-Pro-Leu-Asn-(OH)

20

Seq ID No:55

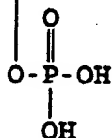
25

This peptide was liberated from the resin and deblocked by treatment as in Example 25. FAB-MS, (M+Na)⁺ calcd., 1064.1; Found, 1063.5.

EXAMPLE 36

Asp-Glu-Tyr-Leu-(OH)

30



Seq ID No:66

35

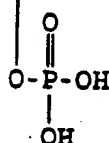
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 617.5; Found, 618.2.

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EXAMPLE 37

Glu-Tyr-Leu-Ile-(OH)

5



Seq ID No:67

10

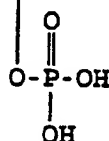
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 624.7; Found, 624.3.

EXAMPLE 38

15

Val-Gln-Asn-Pro-Val-Tyr-His-Asn-Gln-Pro-Leu-Asn-(OH)

20



Seq ID No:68

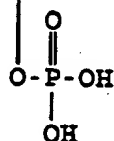
25

This peptide was liberated from the resin and deblocked by treatment as in Example 25. FAB-MS, (M+Na)⁺ calcd., 1501.5; Found, 1501.5.

EXAMPLE 39

Asn-Pro-Val-Tyr-Asn-Gln-Pro-Leu-Asn-(OH)

30



Seq ID No:69

35

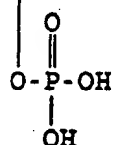
This peptide was liberated from the resin and deblocked by treatment as in Example 25. FAB-MS, (M+Na)⁺ calcd., 1139.3; Found, 1139.2.

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EXAMPLE 40

Pro-Val-Tyr-His-Asn-Gln-Pro-Leu-Asn- (OH)

5



Seq ID No:71

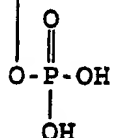
10

This peptide was liberated from the resin and deblocked by treatment as in Example 25.

EXAMPLE 41

Pro-Val-Tyr-Glu-Asn-Pro-Pro-Leu-Asn- (OH)

15



Seq ID No:72

20

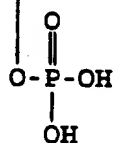
This peptide was liberated from the resin and deblocked by treatment as in Example 25. FAB-MS, (M+Na)⁺ calcd., 1120.1; Found, 1120.3.

25

EXAMPLE 42

Asp-Ala-Asp-Glu-Tyr-Leu- (OH)

30



Seq ID No:73

35

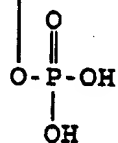
This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 802.3; Found, 803.4.

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EXAMPLE 43

Asp-Ala-Glu-Tyr-Leu-(OH)

5



Seq ID No:74

10

This peptide was liberated from the resin and deblocked by treatment as in Example 1. FAB-MS, (M+Na)⁺ calcd., 688.6; Found, 689.2.

15

Having described the invention, the embodiments thereof in which an exclusive property or privilege is claimed are defined as follows.

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TABLE 1
(Page 1 of 4)

AA=	Example	Seq ID No.
Val-Val-Asp-Ala-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	1	1
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	2	2
Phe-Leu-Pro-Val-Pro-Glu-pTyr-Ile-Asn-Gln-Ser-Val	3	3
Asp-Asn-Pro-Asp-pTyr-Gln-Gln-Asp-Phe-Phe	4	4
Ser-Leu-Asp-Asn-Pro-Asp-pTyr-Gln-Gln-Asp-Phe-Phe	5	5
Ala-Glu-pTyr-Leu-Arg-Val-Ala-Pro-Gln-Ser	6	6
Thr-Ala-Glu-Asn-Ala-Glu-pTyr-Leu-Arg-Val-Ala-Pro-Gln-Ser	7	7
Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Gln-Gly	8	8
Ser-Pro-Gln-Pro-Glu-pTyr-Val-Asn-Gln-Ser-Gly	9	9
Asp-Asn-Leu-pTyr-Tyr-Trp-Asn-Gln-Asn-Ser-Ser	10	10
Asp-Asn-Leu-Tyr-pTyr-Trp-Asp-Gln-Asn-Ser-Ser	11	11
Glu-Asn-Pro-Glu-pTyr-Leu-Gly-Leu-Asp-Val-Pro-Val	12	12
Leu-Thr-Ser-Asn-Gln-Glu-pTyr-Leu-Asp-Leu-Ser-Met-Pro-Leu	13	13
Glu-pTyr-Cys-Tyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Glu-Gln	14	14
Glu-Tyr-Cys-pTyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Glu-Gln	15	15
Ala-Glu-pTyr-Leu-Arg-Val-Ala	16	16
pTyr-Leu-Arg-Val-Ala-Pro-Gln-Ser	17	17
Ala-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	18	18
Asp-Ala-Ala-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	19	19
Asp-Ala-Asp-Ala-pTyr-Leu-Ile-Pro-Gln-Gln-Gly	20	20
Asp-Ala-Asp-Glu-pTyr-Ala-Ile-Pro-Gln-Gln-Gly	21	21
Asp-Ala-Asp-Glu-pTyr-Leu-Ala-Pro-Gln-Gln-Gly	22	22
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Ala-Gln-Gln-Gly	23	23
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Ala-Gln-Gly	24	24
Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Ala-Gly	25	25
Leu-Pro-Val-Pro-Glu-pTyr-Ile-Asn-Ala-Ser-Val	26	26
Glu-pTyr-Leu	27	27
Asp-Glu-pTyr-Leu-Ile	28	28
Ala-Ala-Asp-Ala-pTyr-Ala-Ile-Pro-Ala-Ala	29	29
Asp-Ala-pTyr-Ala-Ile	30	30
	31	31

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TABLE 1
(Page 2 of 4)

AA=	Example	Seq ID No.
Val-Val-Asp-Ala-Asp-Glu-pTyr-Leu		32
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly	23	33
$\begin{array}{c} \text{O} \\ \\ \text{CH}_2\text{-P-O-Me} \\ \\ \text{O-Me} \end{array}$		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		34
$\begin{array}{c} \text{O} \\ \\ \text{CH}_2\text{-P-OtBu} \\ \\ \text{OtBu} \end{array}$		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		35
$\begin{array}{c} \text{O} \\ \\ \text{CF}_2\text{-P-OtBu} \\ \\ \text{OtBu} \end{array}$		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		36
$\begin{array}{c} \text{O} \\ \\ \text{CHF-P-OtBu} \\ \\ \text{OtBu} \end{array}$		

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TABLE 1
(Page 3 of 4)

AA=	Example	Seq ID No.
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly	24	37
$ \begin{array}{c} \text{O} \\ \\ \text{CH}_2\text{-P-OH} \\ \\ \text{OH} \end{array} $		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		38
$ \begin{array}{c} \text{O} \\ \\ \text{CF}_2\text{-P-OH} \\ \\ \text{OH} \end{array} $		
Asp-Ala-Asp-Glu-Phe-Leu-Ile-Pro-Gln-Gln-Gly		39
$ \begin{array}{c} \text{O} \\ \\ \text{CHF-P-OH} \\ \\ \text{OH} \end{array} $		
Leu-Val-Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Glu-Gly		40
Asp-Ala-Asp-Glu-pTyr		41
Ala-Asp-Glu-pTyr-Leu-Ile		42
Ala-Asp-Glu-pTyr-Leu-Ile-Pro		43
Asp-Ala-Ala-Glu-pTyr-Leu-Ala-Ala-Gln-Gln-Gly	33	44
Ala-Ala-Asp-Ala-pTyr-Ala-Ile-Pro-Ala-Ala-Ala	34	45
Asp-Glu-pTyr-Leu		46
Glu-pTyr-Leu-Ile		47
Asp-Glu-pTyr		48
Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Gln-Gly		49
Ser-Pro-Gln-Pro-Glu-pTyr-Val-Asn-Gln-Ser-Glu		50
Glu-pTyr-Cys-Tyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Glu-Glu		51
Glu-Tyr-Cys-pTyr-Asn-Pro-Ser-His-Asn-Pro-Glu-Glu-Gln		52

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TABLE 1
(Page 4 of 4)

AA-	Example	Seq ID No.
Gly-Ser-Val-Gln-Asn-Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	25	53
Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn		54
Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	35	55
Gly-Ser-Val-Gln-Asn-Pro-Val-pTyr-Glu-Asn-Val		56
Pro-Val-pTyr-Glu-Asn-Val		57
pTyr-Ala-Ala-Val	30	58
Leu-Val-Asp-Ala-Glu-Glu-pTyr-Leu-Val-Pro-Gln-Gln-Gly		59
Asp-Ala-Asp-Glu-pTyr	27	60
Ala-Asp-Glu-pTyr-Leu-Ile	28	61
Ala-Asp-Glu-pTyr-Leu-Ile-Pro	29	62
Asp-Ala-Ala-Glu-pTyr-Leu-Ala-Ala-Glu-Glu-Gly	30	63
Ala-Ala-Asp-Ala-pTyr-Ala-Ile-Pro-Ala-Ala-Ala	31	64
Asp-Glu-pTyr-Leu-Ile	32	65
Asp-Glu-pTyr-Leu	36	66
Glu-pTyr-Leu-Ile	37	67
Val-Gln-Asn-Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	38	68
Asn-Pro-Val-pTyr-Asn-Gln-Pro-Leu-Asn	39	69
Asn-Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn		70
Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn	40	71
Pro-Val-pTyr-Glu-Asn-Pro-Pro-Leu-Asn	41	72
Asp-Ala-Asp-Glu-pTyr-Leu	42	73
Asp-Ala-Glu-pTyr-Leu	43	74
Pro-Val-pTyr-Glu-Asn-Pro-Pro-Leu-Asn		75

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CLAIMS

1. A peptide that inhibits the association of a protein tyrosine kinase with a regulatory protein that contains one or more SH2 domains that is selected from one of the following: abl, bcr-abl, GRB2, phospholipase C γ 1 (PLC γ 1), ras GTPase activator protein (GAP), and the p85 subunit of phosphatidylinositol 3' kinase (PI3K); the peptide having an amino acid sequence incorporating the tyrosine autophosphorylation site of a receptor tyrosine kinase in its phosphorylated form or a moiety which mimics the tyrosine autophosphorylation site of a receptor tyrosine kinase in its phosphorylated form that is selected from one of the following: the epidermal growth factor receptor (EGFR), erbB-2/neu growth factor receptor (neu), fibroblast growth factor receptor (FGFR), or macrophage colony stimulating growth factor receptor (CSF-1R).
2. A peptide that inhibits the association of a protein tyrosine kinase with a regulatory protein that contains one or more SH2 domains that is selected from one of the following: abl, bcr-abl, GRB2, phospholipase C γ 1 (PLC γ 1), ras GTPase activator protein (GAP), and the p85 subunit of phosphatidylinositol 3' kinase (PI3K); the peptide having an amino acid sequence incorporating a moiety which mimics the tyrosine autophosphorylation site of a receptor tyrosine kinase in its phosphorylated form that is selected from platelet derived growth factor receptor.
3. A peptide of Claim 1 which is selected from one of the following:

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4. A peptide of Claim 3 which is selected from one of the following:
 - Val-Val-Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly- (OH); Seq ID No:1
 - 5 Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly- (OH); Seq ID No:2
 - Ala-Glu-pTyr-Leu-Arg-Val-Ala- (OH); Seq ID No:16
 - Ala-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly- (OH); Seq ID No:18
 - 10 Asp-Ala-Asp-Ala-pTyr-Leu-Ile-Pro-Gln-Gln-Gly- (OH); Seq ID No:20
 - Asp-Ala-Asp-Glu-pTyr-Ala-Ile-Pro-Gln-Gln-Gly- (OH); Seq ID No:21
 - Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Ala-Gln-Gly- (OH); Seq ID No:24
 - 15 Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Ala-Gly- (OH); Seq ID No:25
 - Asp-Ala-Asp-Glu-pTyr-Leu-Ile-Pro-Gln-Gln-Gly- (OH); Seq ID No:26
 - 20 Gly-Ser-Val-Gln-Asn-Pro-Val-pTyr-His-Asn-Gln-Pro-Leu-Asn- (OH); Seq ID No:53
 - Pro-Val-pTyr-Glu-Asn-Val- (OH); Seq ID No:57
5. A peptide of Claim 3 which is in a conservative mutated form.
6. A peptide of Claim 2 which is in a conservative mutated form.
7. A peptide of Claim 3 wherein the peptide is a N-acetyl and carboxamide terminally modified derivative.
8. A peptide of Claim 2 wherein the peptide is a N-acetyl and carboxamide terminally modified derivative.

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9. A peptide of Claim 3 wherein the moiety which mimics the tyrosine autophosphorylation site of a receptor tyrosine kinase in its phosphorylated form is selected from one of the following:
- 5 (phosphonomethyl)phenylalanine and related derivatives [namely phosphono(α -fluoromethyl)phenylalanine, phosphono(α,α -difluoromethyl)phenylalanine, di-O-(C₁₋₄)alkyl-(α -fluoro)phosphono
- 10 methylphenylalanine, di-O-(C₁₋₄)alkyl-phosphono(α,α -difluoro)methyl-phenylalanine, di-O-(C₁₋₄)alkyl-phosphotyrosine, phosphono(α -hydroxymethyl)phenylalanine, phosphono(α -chloromethyl)phenylalanine], tyrosine
- 15 thiophosphate, N-(C₁₋₄)alkylphosphotyrosines, tyrosine sulfates, 4'-phosphonophenylalanine, aspartic acid, glutamic acid, phosphoserine, and phosphothreonine.
10. A peptide of Claim 2 wherein the moiety which mimics the tyrosine autophosphorylation site of a receptor tyrosine kinase in its phosphorylated form is selected from one of the following:
- 5 (phosphonomethyl)phenylalanine and related derivatives [namely phosphono(α -fluoromethyl)-phenylalanine, phosphono(α,α -difluoromethyl)-phenylalanine, di-O-(C₁₋₄)alkyl-(α -fluoro)-phosphonomethylphenylalanine, di-O-(C₁₋₄)alkyl-
- 10 phosphono(α,α -difluoro)methylphenylalanine, di-O-(C₁₋₄)alkylphosphotyrosine, phosphono-(α -hydroxymethyl)phenylalanine, phosphono-(α -chloromethyl)phenylalanine], tyrosine thiophosphate, N-(C₁₋₄)alkyl phosphotyrosines,
- 15 tyrosine sulfates, 4'-phosphonophenylalanine, aspartic acid, glutamic acid, phosphoserine, and phosphothreonine.

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11. A pharmaceutical composition useful for
controlling proliferative disease such as cancer
and psoriasis and or for treating a viral,
inflammatory, allergic, and/or cardiovascular
disease in a mammal, containing a therapeutically
effective amount of a peptide of Claim 3.
12. A pharmaceutical composition useful for
controlling proliferative disease such as cancer
and psoriasis and or for treating a viral,
inflammatory, allergic, and/or cardiovascular
disease in a mammal, containing a therapeutically
effective amount of a peptide of Claim 2.
13. A method for controlling proliferative disease and
for treating a viral, inflammatory, allergic, and
or cardiovascular disease in a mammal which
comprises administering a topical, oral or
parenteral unit dosage form of a pharmaceutical
composition of Claim 10.
14. A method for controlling proliferative disease and
for treating a viral, inflammatory, allergic, and
or cardiovascular disease in a mammal which
comprises administering a topical, oral or
parenteral unit dosage form of a pharmaceutical
composition of Claim 10. A method for controlling
proliferative disease and for treating a viral,
inflammatory, allergic and or cardiovascular
disease in a mammal which comprises administering
a topical, oral or parenteral unit dosage form of
a pharmaceutical composition of Claim 11.

INTERNATIONAL SEARCH REPORT

Intern. Appl. No.
PCT/US 93/08996

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/06 C07K7/08 A61K37/02 //C12N9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,85 03357 (ICRF PATENTS LTD. ET AL.) 1 August 1985 see claims 30-40 see example 6	1,3,4,6, 10,12
X	WO,A,92 13870 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 20 August 1992 see claims 26-30 see page 49, line 34 - page 52, line 30 -/-	2,5,7, 11,13

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

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- "&" document member of the same patent family

Date of the actual completion of the international search

28 January 1994

Date of mailing of the international search report

04 -03- 1994

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Fax (+31-70) 340-3016

Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/08996

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLECULAR AND CELLULAR BIOLOGY vol. 11, no. 2 , February 1991 , WASHINGTON DC, USA pages 1125 - 1132 J. ESCOBEDO ET AL. 'A phosphatidylinositol-3 kinase binds to platelet-derived growth factor receptors through a specific receptor sequence containing phosphotyrosine.' cited in the application see abstract see table 1 ----	2,5,7
P,A	CA,A,2 054 602 (MOUNT SINAI HOSPITAL CORPORATION) 1 May 1993 see the whole document ----	1-13
P,X	MOLECULAR AND CELLULAR BIOLOGY vol. 13, no. 3 , March 1993 , WASHINGTON DC, USA pages 1449 - 1455 S. FELDER ET AL. 'SH2 domains exhibit high-affinity binding to tyrosine-phosphorylated peptides yet also exhibit rapid dissociation and exchange.' see abstract see table 2 ----	1,2
P,X	ANNUAL REPORT OF MEDICINAL CHEMISTRY vol. 27 , 1992 , WASHINGTON DC, USA pages 169 - 178 E. DOBRUSIN ET AL. 'Chapter 18. Protein tyrosine kinases and cancer.' see page 175, line 27 - page 176, line 2 -----	1,2,9, 11,13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/08996

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 12 and 13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The present set of claims contains two times a claim numbered "4"
Claims 3 and 4 have been interpreted and searched for as follows :
Page 49 lines 1 and 2 should be erased. / Claim 3 : page 48 lines 1 & 2
and page 49 lines 4-16. / Claim 4 : page 49 lines 18 & 19.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/08996

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-8503357	01-08-85	AU-A- 3934085 DE-D- 3587657 EP-A- 0171407 EP-A- 0491675 JP-T- 61501168 US-A- 4933294	09-08-85 23-12-93 19-02-86 24-06-92 12-06-86 12-06-90
WO-A-9213870	20-08-92	AU-A- 1346792 EP-A- 0572505	07-09-92 08-12-93
CA-A-2054602	01-05-93	NONE	